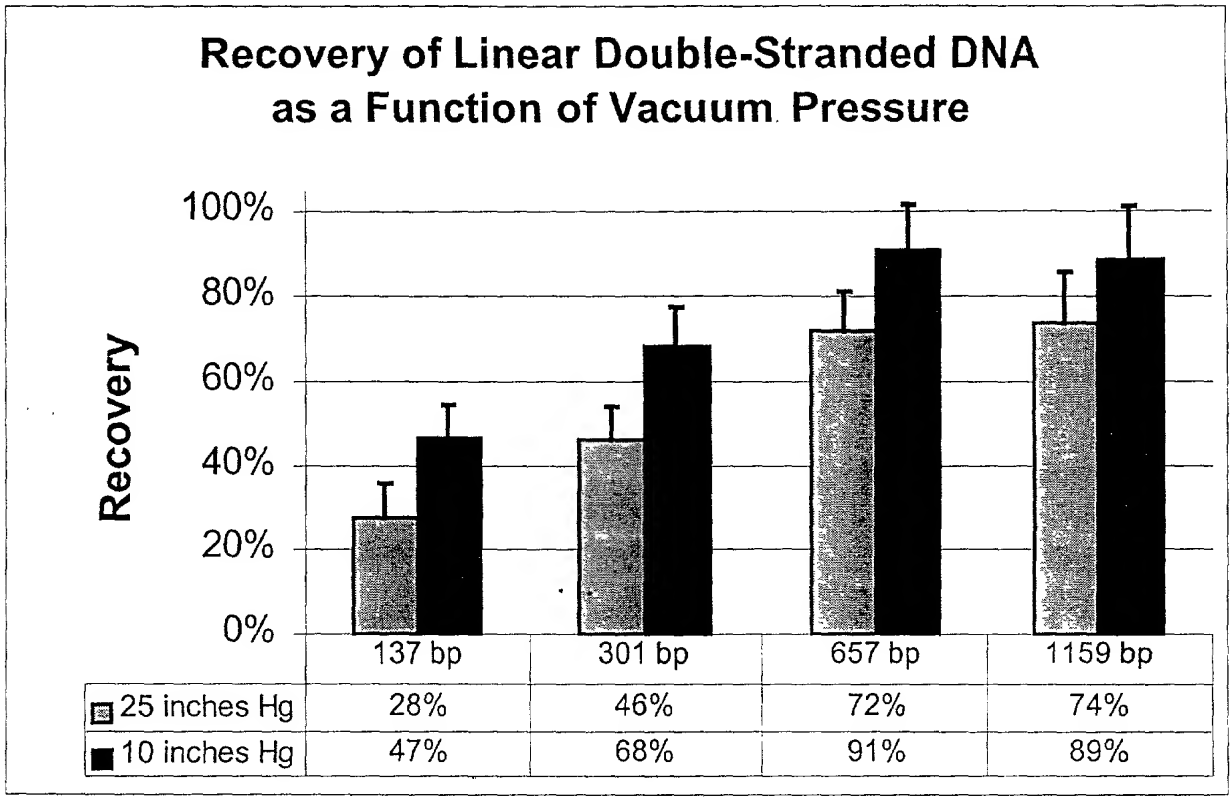


Figure 1. **Recovery of Linear Double-Stranded DNA as a Function of Vacuum Pressure**
Increased fractionation between small and large linear double-stranded nucleic acids is achievable by continuous pressure differential ultrafiltration at increased vacuum pressure (e.g., 25 inches Hg). Thus, increased recovery of smaller linear double-stranded is achievable by ultrafiltration at reduced vacuum pressure (e.g., 10 inches Hg). Twenty microliter PCR reactions of increasing product sizes (n=96, each product at each operational condition) were purified using MultiScreen-384-PCR plates, filtered to dryness either 15 or 25 minutes at 25 or 10 inches Hg, respectively. Purified samples were resuspended in 100 microliters of TE buffer and percent mass recovery was determined by concentration versus unpurified starting material using a fluorescence-based solution assay with SYBR Green I nucleic acid stain (Molecular Probes, Eugene, OR).

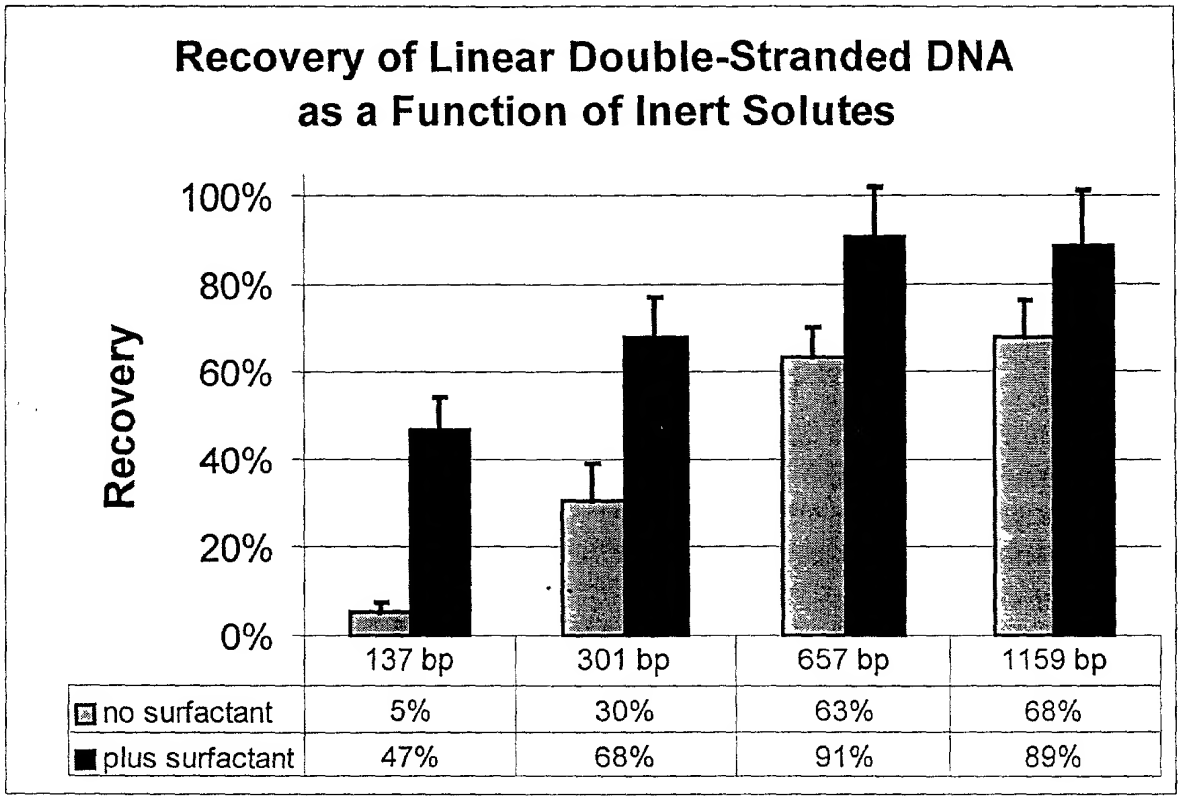


Figure 2. **Recovery of Linear Double-Stranded DNA as a Function of Inert Solutes**
Inert polarizing solutes decrease fractionation and thereby enhance recovery of small linear double-stranded nucleic acids during continuous pressure differential ultrafiltration. PCR products of increasing sizes were generated using reaction buffers from different vendors, which primarily differ by the presence of the surfactant Triton X-100 at 0.1% final concentration. Twenty microliter PCR reactions (n=96, each product in each buffer) were purified using MultiScreen-384-PCR plates, filtered 15 or 25 minutes at 10 inches Hg, minus or plus surfactant, respectively. Purified samples were resuspended and percent mass recovery was determined using the SYBR Green I recovery assay.

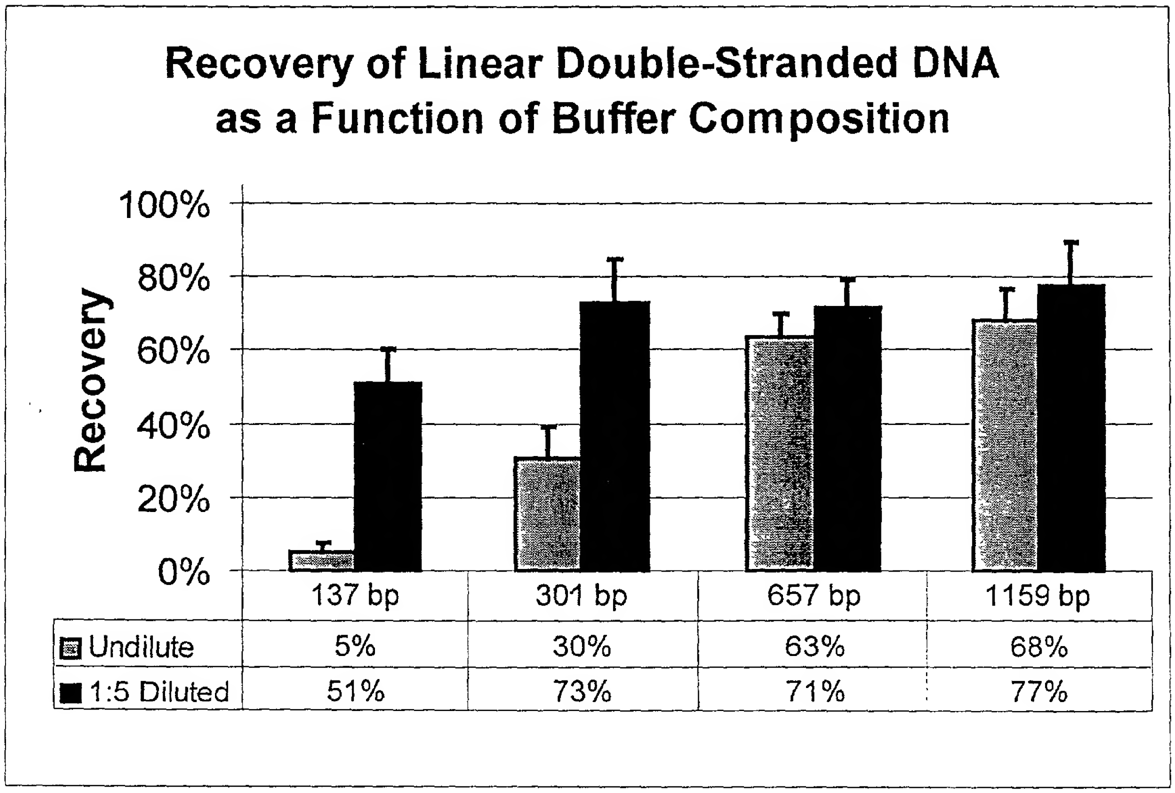
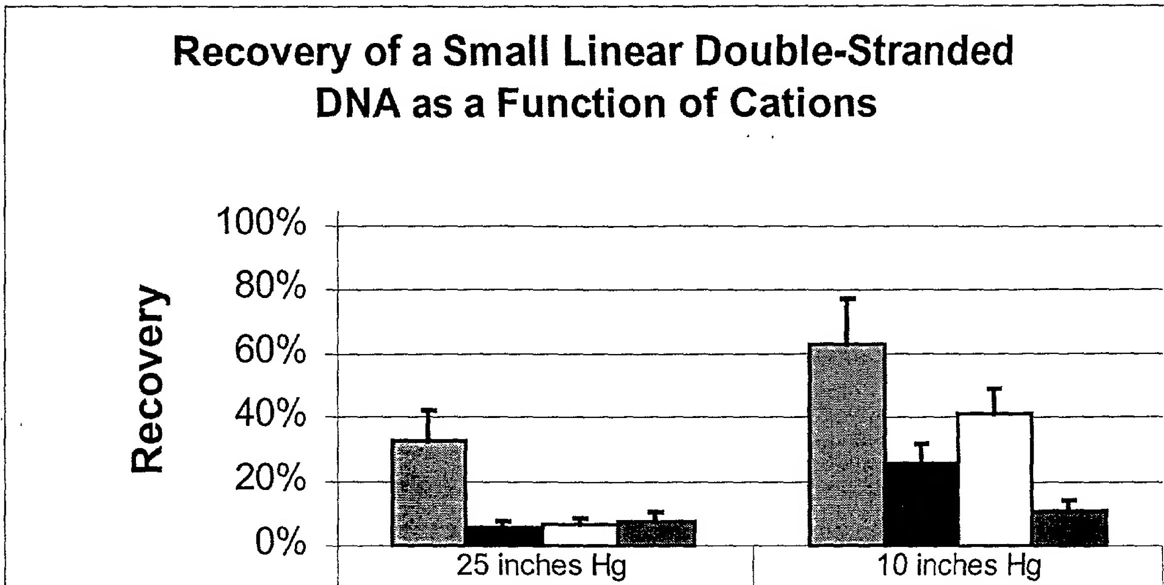


Figure 3. **Recovery of Linear Double-Stranded DNA as a Function of Buffer Composition**
In the absence of flux-governing inert solutes (e.g., surfactant), enhanced recovery of smaller linear double-stranded nucleic acids is achievable via sample dilution, which paradoxically increases filtration rate. The effect is therefore the result of diluting buffer components rather than reducing solute concentration (supported also by Example 6). Thus, increased fractionation of linear double-stranded nucleic acids is achievable under increasing salt conditions. Twenty microliter PCR reactions (n=96, each product undiluted) were purified using MultiScreen-384-PCR plates filtered for 15 minutes, and 100 microliter 1:5-diluted PCR products (n=96, each product diluted in TE buffer) were filtered 20 minutes at 10 inches Hg. Purified samples were resuspended and percent recovery was determined using the SYBR Green I recovery assay.



	25 inches Hg	10 inches Hg
TE buffer	33%	63%
50 mM KCl	6%	26%
1.5 mM MgCl2	7%	41%
KCl + MgCl2	8%	11%

Figure 4. **Recovery of a Small Linear Double-Stranded DNA as a Function of Cations**
The effect of salts on fractionation and recovery of linear double-stranded nucleic acids is dependent on both operational vacuum pressure and salt concentration. Using MultiScreen-PCR plates (96-well), 50 microliter PCR reactions of a 137 bp product were diluted 1:3 using different diluents (as indicated in the example; n=24, each diluent at each pressure) and purified by filtration to dryness at either 25 or 10 inches Hg vacuum pressure. Samples were resuspended in 50 microliters TE buffer and percent recovery was determined using the SYBR Green I recovery assay. As shown in the example, the effect of salt concentration on fractionation (as measured by recovery) is more pronounced at higher operational vacuum pressure. At the lower vacuum pressure, the differential effects of 50 mM potassium chloride and 1.5 mM magnesium chloride indicate that salt effects are a function of cationic species and concentration rather than being an anionic effect. A greater degree of fractionation is achievable with far less divalent cation (magnesium) than using a monovalent cation (potassium), whereas their combined effects are additive.

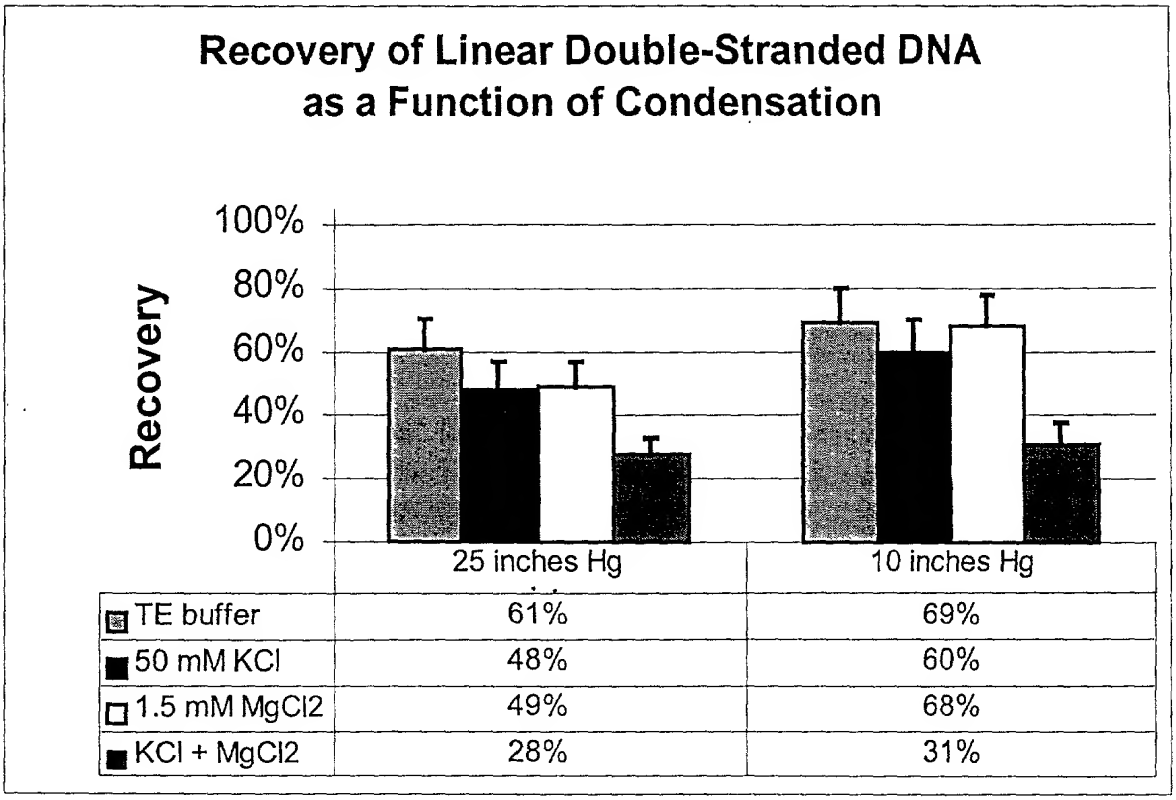


Figure 5. **Recovery of Linear Double-Stranded DNA as a Function of Condensation**
The effect of salt on the fractionation of nucleic acids during continuous pressure differential ultrafiltration is dependent on fragment length and vacuum pressure. Using MultiScreen-PCR plates (96-well), 50 microliter PCR reactions of a 301 bp product were diluted 1:3 using different diluents (as indicated in the example; n=16, each diluent at each pressure) and purified by filtration to dryness at either 25 or 10 inches Hg vacuum pressure. Samples were resuspended in 50 microliters TE buffer and percent recovery was determined by concentration versus unpurified starting material by gel electrophoresis, ethidium bromide staining and densitometric scanning. As shown in the example, additive effects of monovalent and divalent cations are observed at high vacuum pressure, whereas synergistic effects are observed at lower pressure. These data indicate that the effect of the divalent cation, magnesium is likely a result of molecular condensation rather than simply salting out the phosphate backbone of the DNA, as the greater concentration of potassium would easily achieve in the presence or absence of the magnesium.

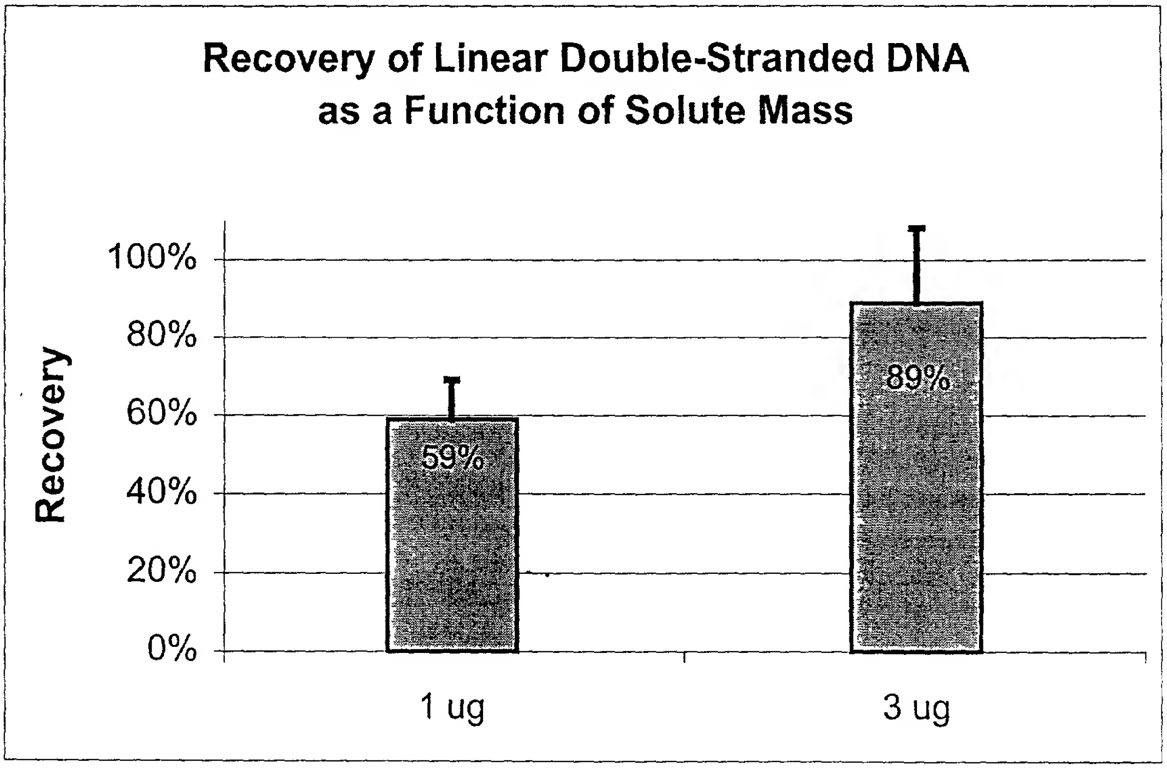


Figure 6. Recovery of Linear Double-Stranded DNA as a Function of Solute Mass
A 301 bp PCR product that had been amplified for extended rounds and yielded three-times higher product was purified using MultiScreen-PCR, and was compared to purification of the typical yield 301 bp product. Fifty microliter undiluted PCR reactions (n=16, each) were purified by filtration to dryness at 25 inches Hg vacuum pressure. Samples were resuspended in 50 microliters TE buffer and percent recovery was determined by concentration versus unpurified starting material by gel densitometry.

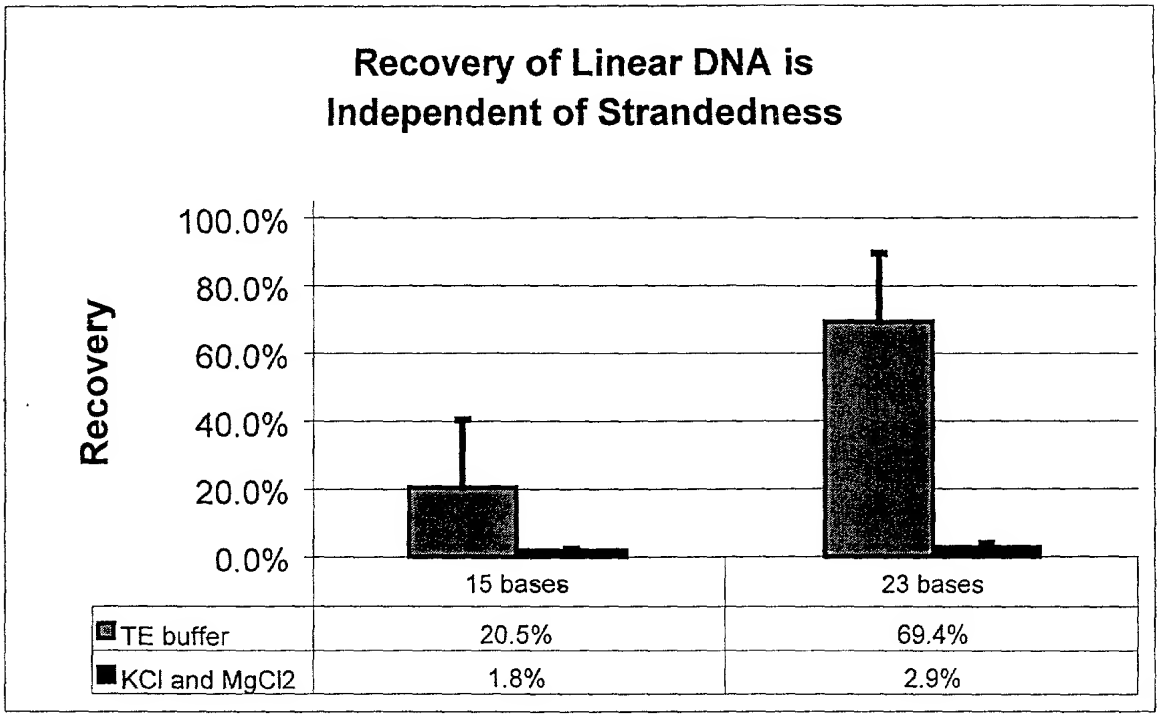


Figure 7. **Recovery of Linear DNA is Independent of Strandedness**
The behavior of nucleic acids in continuous pressure differential ultrafiltration is not limited to double-stranded fragments. Short, single-stranded oligonucleotides exhibit similar fractionation properties in the presence of salts. Different length fluorescent-labeled oligonucleotides were filtered from 20 microliters to dryness using a MultiScreen-384-SEQ plate, in TE buffer or in a 1:2 diluted mock mini-sequence reaction (template-free; 5 mM Tris-HCl, pH 8.3, 15 mM potassium chloride, 1.5 mM magnesium chloride, 25 uM each dNTPs). Samples were resuspended in 100 microliters of TE buffer and percent recovery versus each input oligonucleotide was determined by relative fluorescence (n=96, each).

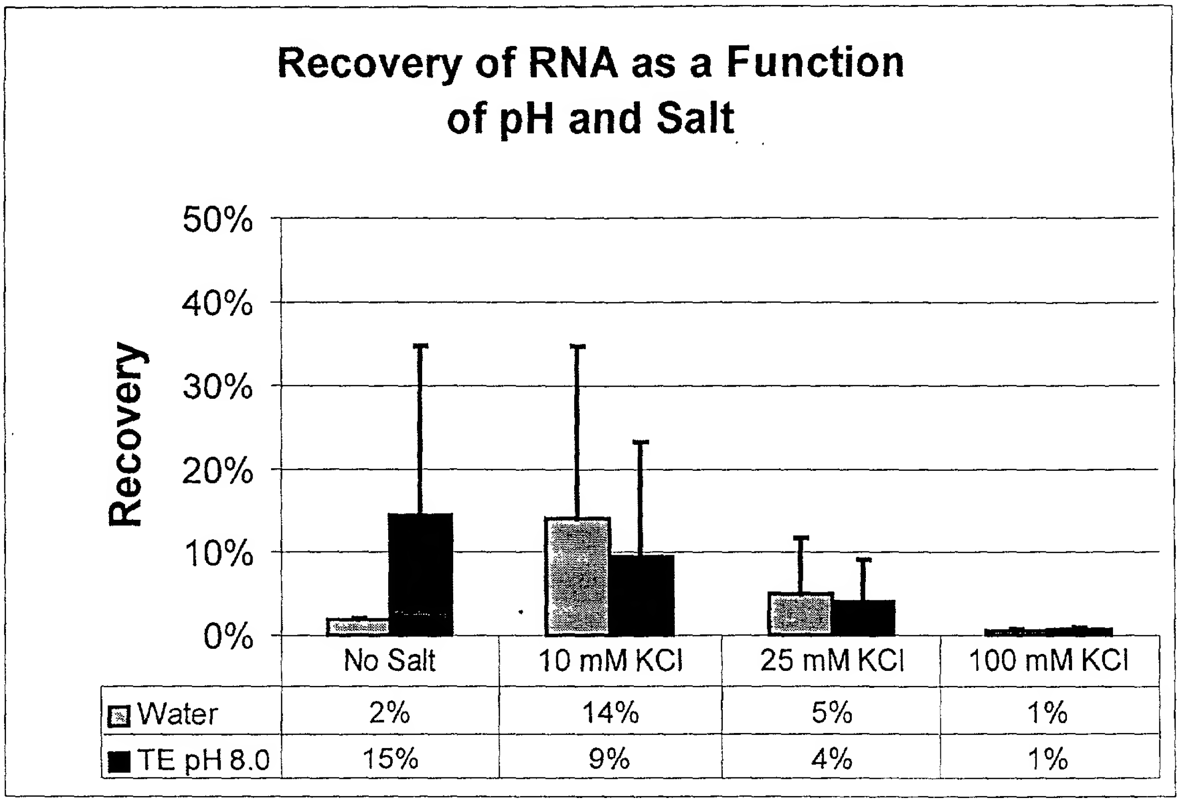


Figure 8. **Recovery of RNA as a Function of pH and Salt**

The behavior of nucleic acids in continuous pressure differential ultrafiltration is not limited to DNA fragments. Fractionation and recovery of RNA by vacuum filtration is similarly affected by salt concentration. Transfer RNA (~70-80 bases) was diluted in increasing salt-containing solutions, either based in water or TE buffer, pH 8.0, prior to filtering 500 ng per well using two MultiScreen-384-SEQ plates. Buffer alone, versus water, enhances recovery of the tRNA to a similar extent as 10 mM salt in water. These data suggest that the effect of the TE on tRNA recovery is likely to be a salt affect of introducing base pairing in the RNA secondary structure, but that greater concentrations of total salt have an inverse effect on recovery. However, these data do not exclude the possibility that the effect of TE is pH-based. Nonetheless, increasing salt concentration increases RNA fractionation potential and thereby decreases recovery after continuous pressure differential ultrafiltration. RNA samples were introduced in 100 microliters, filtered to dryness, resuspended in 100 ul water or TE buffer, and percent recovery was determined using the SYBR Green I recovery assay.